

# Renal Chemoreceptors in the Rat

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**SUMMARY** There are afferent nerve fibers responsive to alterations of the kidney's chemical environment in the renal nerves of the rat. In anesthetized, artificially ventilated, male Sprague-Dawley rats, single unit recordings were prepared by dissection of the centrally cut nerves of the right kidney. The stimuli used included occlusion of the renal artery, systemic asphyxia, changes in renal arterial and venous pressures, changes in ureteral pressure, and cyanide infusion. We found a population of sensory nerve fibers whose endings are activated only during markedly impaired renal blood flow (produced by clamping the renal artery, severe hypotension below 40 mm Hg, and prolonged occlusion of the renal vein), and during systemic asphyxia. The same units are not responsive to increases and decreases in systemic arterial pressure (range: 40-190 mm Hg), to ureteral pressure (range: 0-50 mm Hg), or to changes in renal venous pressure. None of the 40 single units studied was spontaneously active; their pattern of activation during renal ischemia always was characterized by trains of impulses. These sensory units have functional properties distinctly different from those of known renal mechanoreceptors. They appear to be a homogeneous group of sensory elements, and we have termed them renal ("R") chemoreceptors. Evidence also is presented which is consistent with the concept that a chemical substance released by or accumulated within the kidney might be the agent activating these chemoreceptors during renal ischemia.

**ACTION** potentials initiated by the activation of sensory nerve endings in the kidney have been recorded from the renal nerves of rats,<sup>1</sup> cats,<sup>2-4</sup> dogs,<sup>5</sup> and rabbits.<sup>6-8</sup> The renal receptors thus disclosed respond only to changes in intrarenal pressure and have been termed renal mechanoreceptors. In two studies, occlusion of the renal artery was observed to activate a group of fibers different from those sensitive to intrarenal pressure;<sup>2,3</sup> however, no attempts were made to characterize this group further.

The present report describes a population of afferent fibers from the kidney responsive to renal

ischemia but not to changes in intrarenal pressure. We found suggestive evidence that the stimulus responsible for their activation is the release or accumulation of a chemical substance inside the ischemic kidney. To underline their peculiar functional properties and to differentiate them from the well-known carotid and aortic body chemoreceptors, we have termed the renal nerve endings sensitive to ischemia renal ("R") chemoreceptors.

## Methods

We studied 50 male Sprague-Dawley rats (150-250 g) bred in our laboratories. They were anesthetized by intraperitoneal injection of sodium pentobarbital, 5 mg/100 g (40 rats), or Inactin, 12.5 mg/100 g (10 rats). After a paralyzing dose of gallamine triethiodide, 2 mg/100 g, iv, the rats were ventilated artificially. The respirator was adjusted to maintain arterial  $P_{O_2}$ ,  $P_{CO_2}$ , and pH within normal limits as tested with a Radiometer blood gas analyzer (BMS 3 MK2). The rats were maintained at 37°C on a heated operating table.

Polyethylene catheters were inserted into (1) an

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external jugular vein for continuous infusion of 0.9% NaCl solution at 40  $\mu$ l/min and intermittent sustaining doses of gallamine triethiodide and anesthetic, (2) the femoral and carotid arteries for continuous monitoring of arterial blood pressure, (3) the vena cava, by way of a femoral vein, to monitor venous pressure continuously, and (4) the right ureter close to the renal pelvis. Arterial, venous, and ureteral pressures were measured with Statham P23Db strain gauges and recorded on a Hewlett-Packard 7414-A polygraph recorder.

The rats were placed on their left side and the right kidney was exposed retroperitoneally through a paravertebral incision. A short section of the renal artery, the abdominal aorta below the emergence of the renal artery, and the inferior vena cava above and below the renal vein were carefully cleared of connective tissue, to allow complete occlusion by silk tourniquet or clamping with forceps during the experiment. In four rats, the thoracic aorta was exposed so that renal ischemia could be induced by occluding it, thereby preventing distortion of the renal artery. In nine experiments, a 30-gauge needle was inserted into the renal artery with the tip pointing in the direction of the blood flow. The needle was connected through a polyethylene catheter to an infusion pump (model 355, Sage Instruments).

Increases in renal perfusion pressure were induced either by constriction of the abdominal aorta below the renal arteries or by injection of norepinephrine (1  $\mu$ g, Levophed, Winthrop) into the inferior vena cava. Renal venous pressure was raised by directly clamping the renal vein or by occlusion of the inferior vena cava directly above and below the emergence of the right renal vein. This method of venous occlusion allowed complete block of renal venous outflow while renal venous pressure was monitored. Ureteral pressure was raised in three ways: (1) by elevating the long (50-cm) ureteral cannula to increase hydrostatic pressure to known values (measured as the height of the column of fluid above the kidney level); (2) by plugging the ureteral cannula into a pressure transducer connected to an adjustable reservoir containing isotonic saline; and (3) by connecting the ureteral cannula to a pressure transducer while isotonic saline was infused iv at 0.5 ml/min to cause extracellular volume expansion. Methods 2 and 3 allowed continuous monitoring of ureteral pressure changes.

### Analysis of Neural Activity

The renal nerves were dissected from the surrounding tissues on each side of the renal artery, from the renal hilus to their junction with the coeliac ganglion, then cut centrally and positioned on bipolar hook electrodes. The dissection and recording techniques have been described elsewhere.<sup>9</sup> The greater splanchnic and all other visible nerves joining the coeliac plexus were severed, to isolate the right kidney from efferent neural influences.

Nerve activity and hemodynamic variables were stored on a magnetic tape recorder (Ampex FR 1300) and subsequently analyzed with the aid of a computer program<sup>10</sup> that permitted analysis of the individual activity of up to four simultaneously recorded units. Figure 1 shows an example of such an analysis. The analog recording of the activity of two units (Fig. 1, A-E) was stored, after digital conversion, in the computer memory. The spikes then could be displayed on a monitor (Fig. 1F), using a time allocation of 3.4 msec for each spike. The electrical activity of each axon could be distinguished on the basis of the shape and amplitude of the action potentials and thus categorized (numbers 1 and 2 at the bottom of Fig. 1F). Quantitative descriptions were based on plots of instantaneous frequency ( $1/DT = 1/\text{interval between successive impulses}$ , measured in impulses per second) and histogram plots of the number of impulses occurring every 2 (imp/2 sec) and 5 seconds (imp/5 sec).

Quantitative data are expressed as mean  $\pm$  SEM. Statistical comparisons were made by paired Student's *t*-test when appropriate.

## Results

### Identification of Chemoreceptive Afferent Fibers

Responses to renal ischemia and to systemic asphyxia were the criteria for identifying chemoreceptive fibers; renal mechanoreceptors are known to be unresponsive to these stimuli.<sup>1-8</sup> A small renal nerve bundle, which under control conditions was not spontaneously active, gave responses to these events as shown in Figure 2: 35 seconds after the beginning of renal artery occlusion (Fig. 2A, RAO) a few fibers became active almost simultaneously, reaching a peak firing rate (above 300 imp/sec) 7 seconds later; the afferent activity ceased completely 3-4 seconds after the release of renal artery occlusion. Figure 2B shows the response of the same nerve bundle to systemic asphyxia; it was recorded 9 minutes after the end of the record shown in Figure 2A. At the first arrow (Fig. 2B), gallamine was injected iv and the rat stopped breathing; the afferent activity appeared 64 seconds later and progressively diminished during the rise in systemic blood pressure which followed the start of artificial ventilation (second arrow).

The nerve bundles containing fibers responsive to renal ischemia subsequently were dissected in order to record the activity of single units. Since we could differentiate the responses of two or three units recorded simultaneously in the same nerve strand, it often was possible to compare the responses of both a unit sensitive to renal ischemia and a unit sensitive to changes in intrarenal hydrostatic pressure during the same experimental interventions.

Mechanically excitable renal afferent fibers were classified as arterial, venous, or ureteral receptors

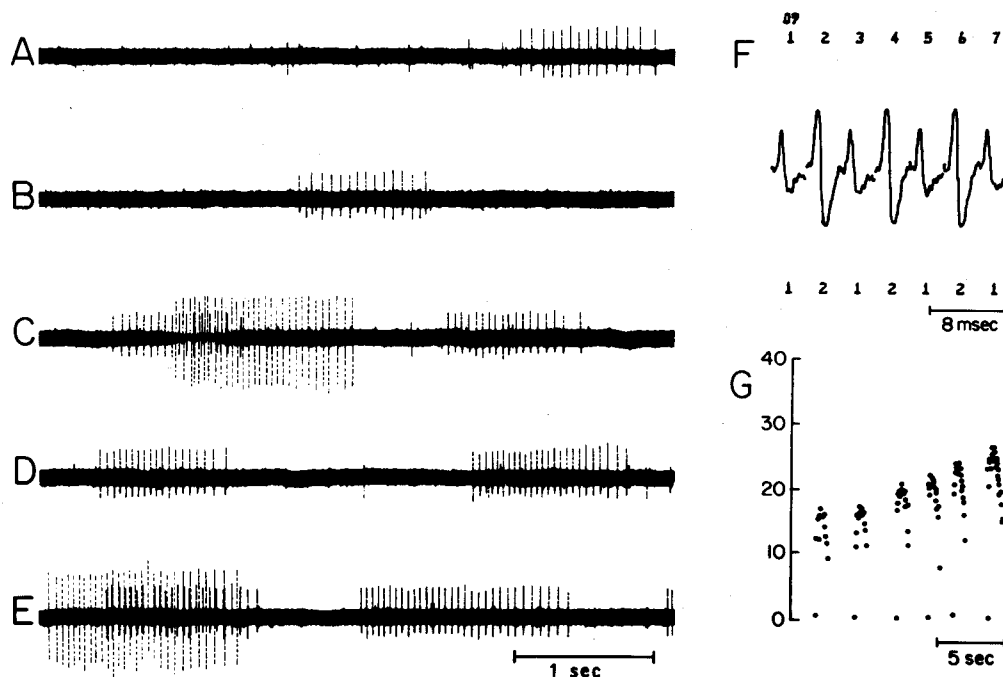


FIGURE 1 Continuous analog recording of the action potentials of two units activated during renal artery occlusion. The occlusion was started 24 seconds before the appearance of the first train of impulses in A. A different unit characterized by a larger action potential was activated 6-7 seconds later (C). All the single units studied became active with a similar pattern of discharge, i.e., trains of impulses. F: Monitor display of the action potentials of the two units shown in A-E, after computer processing. Numbers above the spikes indicate their progressive number in the run being examined. Numbers below identify action potentials as belonging to unit 1 or 2. G: Computer display of the instantaneous frequency of discharge of the first six trains of impulses of unit 1. The position of each dot along the ordinate is the reciprocal of the interval between each spike and the previous one. In all the following figures, the instantaneous firing rate has been represented in similar fashion.

on the basis of their responses to increments in arterial, venous, and ureteral pressure, but no quantitative evaluation of their responses was attempted.

### Effects of Renal Ischemia and Systemic Asphyxia

The responses of two single units were recorded simultaneously from the same nerve bundle, during renal ischemia (Fig. 3A, RAO) and systemic asphyxia (Fig. 3B, ASP); a partial analog recording of the same units appears in Figure 1, A-E. Neither was spontaneously active. Unit 1 was activated 30 seconds and unit 2 25 seconds after the beginning of renal artery occlusion, yielding bursts of impulses that repeated at a frequency different for each unit (Fig. 1, A-E). At the release of the occlusion, the afferent discharge ceased almost immediately. All 40 units studied during renal artery occlusion shared two features: (1) none was spontaneously active, and (2) when activated, each responded with trains of impulses (Fig. 1, A-E). The average latency of the response to renal ischemia produced by occlusion of the renal artery was  $38.7 \pm 3.3$  sec (mean  $\pm$  SE; range: 11-66). The average peak frequency reached during the occlusion was  $32.7 \pm 7.0$

imp/sec (range: 14-48). Some units reached their peak firing rate with the first burst of impulses (Fig. 3A, unit 1); others took longer (Fig. 3A, unit 2).

Systemic asphyxia was produced either by injecting gallamine into spontaneously breathing rats (7 units) or by interrupting artificial ventilation (13 units). In both cases it was followed by a very brief period of hypertension before an abrupt fall in systemic blood pressure (Figs. 2B and 3B). Activation of afferent nerve fibers always occurred during the hypotensive phase, and the average latency after respiratory arrest was  $77.5 \pm 13.4$  sec. (range: 20-128). The average peak frequency was similar to that reached during renal artery occlusion and when renal perfusion pressure was maintained below 40 mm Hg during hemorrhage (see below). Figure 3B shows how asphyxia produced by interrupting artificial ventilation affected the two units of Figure 3A and Figure 1, A-E. They became active with bursts of impulses after 50 seconds (unit 1) and 44 seconds (unit 2); activation ceased as the systemic blood pressure rose at the resumption of artificial ventilation.

The response to renal artery occlusion displayed by 10 units studied in the spontaneously breathing rat was the same when the rats later were paralyzed

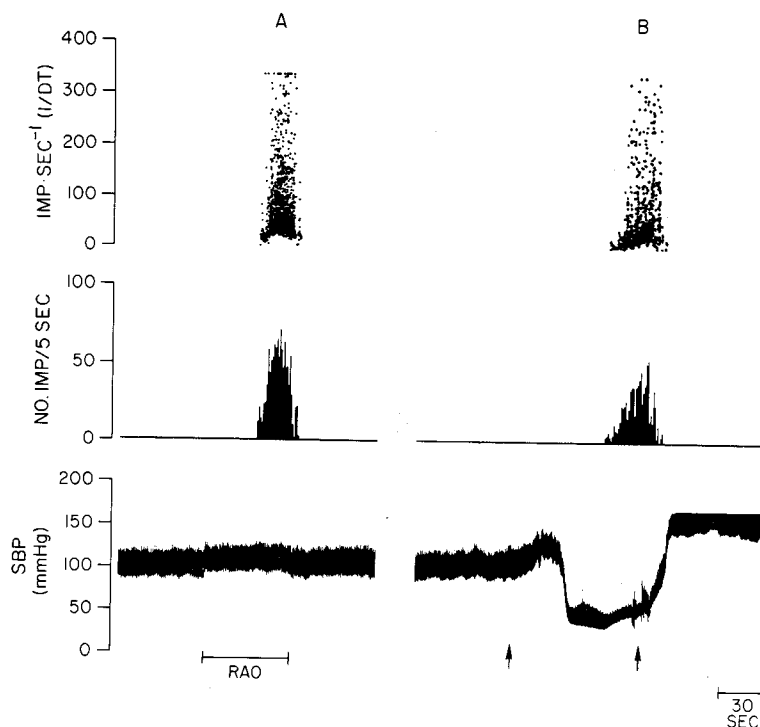


FIGURE 2 Effects of renal artery occlusion (A, RAO) and systemic asphyxia (B, between the arrows) on a small multifiber preparation which in control conditions was not active. The rat was breathing spontaneously. Traces from top to bottom are: instantaneous frequency of discharge, number of spikes each 5 seconds, and systemic blood pressure (SBP). A: The afferent activity appeared 35 seconds after the beginning of renal artery occlusion. The frequency of discharge reached the saturation frequency of the computer program (333 imp/sec). The activation ceased a few seconds after the occlusion was released. B: The activation began 24 seconds after the injection of gallamine iv (first arrow) and it ceased about 10 seconds after the respiratory pump was started (second arrow).

and artificially ventilated. Moreover, whether the anesthetic employed was sodium pentobarbital (40 experiments) or Inactin (10 experiments), there was no difference in the response.

#### Prolonged Renal Ischemia

The effects of a prolonged (3-minute) occlusion of the renal artery were studied in five experiments (five units). The mean duration of the discharge was  $81.4 \pm 13.8$  sec. The frequency of the discharge initially increased, then declined to low levels, and finally ceased completely, despite the persistence of total renal ischemia. In contrast, a long-lasting activation occurred after the rat had been killed by injection of pentobarbital or by asphyxia. In five experiments, afferent fibers responsive to ischemia were still active 25–30 minutes after death.

#### Repeated Occlusions of the Renal Artery

On repeated occlusion of the renal artery, the responses of a given unit remained constant in latency and frequency of discharge, provided that the interstimulus intervals exceeded 10 minutes. At shorter intervals, the latency increased for the first repetition and the peak firing rate decreased.

Figure 4 shows how two series of five consecutive occlusions, repeated at 1-minute intervals, affected the mean latency of seven units (seven experiments). In the first series, occlusion lasted 1 minute; in the second series, 2 minutes. Ten- to 20-minute recovery intervals separated the two series. In both series, the latency of the response to the second occlusion was significantly longer than for the first ( $P < 0.01$ , paired *t*-test); the third, fourth, and fifth

latencies resembled the second. Although the interstimulus interval was the same (1 minute) in both series, the second and subsequent occlusions of the 2-minute series evoked responses at longer latencies than in the 1-minute series. The latency of the response to renal artery occlusion thus is affected not only by the time interval between occlusions but also by the duration of the preceding period of renal ischemia.

#### "Wash-out" Effect

The release of the renal artery occlusion invariably led to an immediate cessation of activity, and isotonic saline perfused through the ischemic kidney stopped the activation as effectively as reentering blood. In five experiments, a needle inserted into the renal artery was connected to an infusion pump via a polyethylene cannula, and a thread was tied around the renal artery proximal to the needle to make the kidney ischemic. Figure 5 shows a typical result. After the artery had been tied, two units became active with bursts of impulses. Saline injection began (1 or 2 ml/min), whereupon unit 2 stopped firing and the activity of unit 1 decreased markedly. The afferent activity reappeared a few seconds after the perfusion was stopped and ceased completely on release of the occlusion.

The oxygen content of the saline ( $P_{O_2} = 120$  mm Hg) was immaterial to the inhibition of the response. In two experiments, the ischemic kidney was perfused with deoxygenated saline ( $P_{O_2} = 20$ – $30$  mm Hg after 30 minutes of bubbling with helium gas); a complete inhibition of the afferent activity resulted (two units). In two additional experiments,

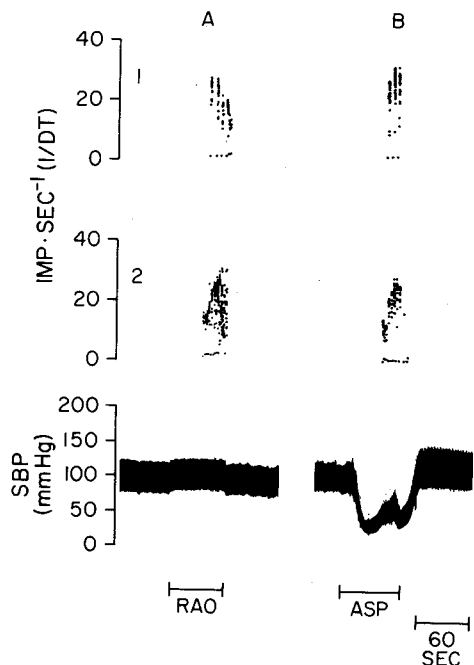


FIGURE 3 Effects of renal artery occlusion (A, RAO) and systemic asphyxia (B, ASP) on the activity of two units (1 and 2) recorded simultaneously from the same nerve bundle. Traces from top to bottom are: instantaneous firing rate of units 1 and 2, and systemic blood pressure (SBP). Unit 1 showed a longer latency and a slower frequency of repetition in the trains of impulses. The activation of both units ceased immediately on the release of the occlusion and at the resumption of artificial ventilation.

infusion of isotonic mannitol also produced a complete cessation of the afferent activity.

### Effects of Mechanical Stimuli

#### Changes in Arterial Blood Pressure

Increments in arterial blood pressure were caused by occlusion of the abdominal aorta below the renal artery (three experiments, four units) and by injections of norepinephrine (five experiments, six units). None of the 10 chemoreceptive units studied was activated. Systolic blood pressure increased from an average control value of  $111.5 \pm 5.7$  to  $146.2 \pm 5.7$  mm Hg with such an occlusion and from  $108.6 \pm 4.7$  to  $186.4 \pm 7.5$  mm Hg with norepinephrine.

Lowering arterial blood pressure by bleeding was studied in 10 experiments (12 units). Figure 6 shows the effect of a slow hemorrhage on two units sensitive to renal ischemia. The first arrow indicates the start of blood withdrawal through a catheter placed in the inferior vena cava, to reduce the systolic blood pressure to 70 mm Hg (2 ml withdrawn). After 80 seconds (second arrow), another milliliter of blood was withdrawn to lower the blood pressure to 50 mm Hg; no units were activated. At the third

arrow, more blood was slowly extracted until the fibers became active (total hemorrhage: 4.6 ml). The blood was slowly reinjected into the rat (upward arrow), and the afferent fiber activity progressively decreased as the blood pressure rose. The average threshold for lowered pressure in the 12 units studied was  $34.8 \pm 3.2$  mm Hg (range: 15–40 mm Hg).

Thus, changes in arterial blood pressure in the range of 40–190 mm Hg do not activate the afferent fibers responsive to renal ischemia. In contrast, renal mechanoreceptors are known<sup>6-8</sup> to be activated by increases in blood pressure, whereas their discharge ceases during lowering of blood pressure by hemorrhage.

#### Changes in Venous Pressure

Occlusion of the renal vein was studied, first, to determine whether increments in venous pressure activate the receptors and, second, to compare the effects of renal ischemia produced by renal venous stasis (with its concomitant of high intrarenal pressure) to those of renal ischemia produced by occlusion of the renal artery.

Figure 7 shows how clamping the renal vein affected a single unit that responded to a brief occlusion of the renal artery after a 32-second latency. A venous occlusion was performed a few minutes after the release of the previous arterial occlusion; 53

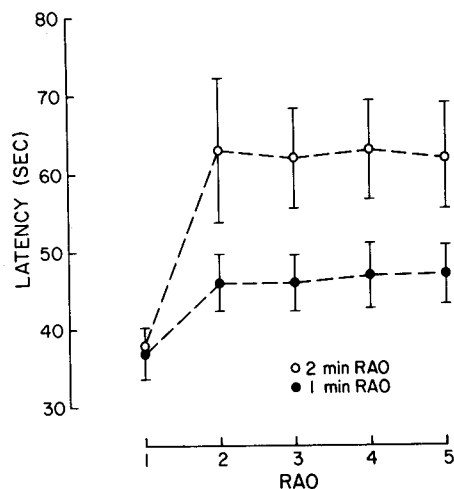


FIGURE 4 Effects of repeated renal artery occlusion on the average latency of the response of seven units (mean  $\pm$  SE). Each unit was tested during two series of five occlusions of the renal artery; the two series were separated by a 10- to 20-minute interval. In one series, the occlusions were maintained for 1 minute (filled circles) and in the other for 2 minutes (unfilled circles). In each series, the interval between one occlusion and the next was 1 minute. Note that the average latency at the first occlusion in both series was very similar, and the latency at the second was significantly different from the first ( $P < 0.01$ ). The 2-minute occlusions had a significantly longer latency than the 1-minute ( $P < 0.001$ ).

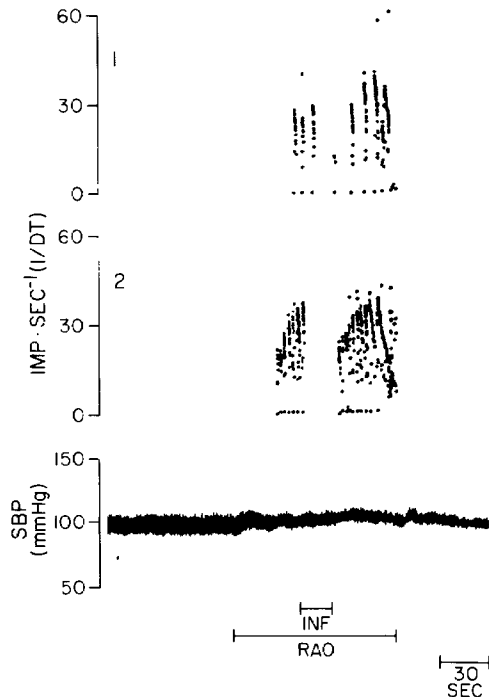


FIGURE 5 "Wash-out" effect. Traces as in Figure 3. Renal ischemia (RAO) was produced by tying a thread around the renal artery proximal to a previously inserted 30-gauge needle. The activity of two units elicited by renal ischemia ceased a few seconds after the start of an infusion of isotonic saline into the ischemic kidney (INF). The activity reappeared after the infusion was stopped and ceased again at the release of the occlusion. The reperfusion of the ischemic kidney with isotonic saline therefore had the same inhibitory effects as reentry of blood at the release of the occlusion. During this trial, the pulsatility of the systemic blood pressure trace became damped.

seconds later the unit produced the characteristic bursts of impulses. Five other units activated by the venous occlusion at a mean latency of  $65 \pm 7.5$  sec had a mean latency of  $42.8 \pm 10.1$  sec for the arterial occlusion. The longer latency of the response to venous occlusion suggests that increments in venous pressure per se were not responsible for the activation. Had these fibers been sensitive to increments in venous pressure, their response to a complete occlusion of the renal vein should have been almost immediate, as is the case for renal mechanoreceptors.<sup>4</sup>

The long latency did not, however, completely preclude mechanosensitivity in the units under study: their threshold to intrarenal pressure changes might have been very high. To investigate this possibility, the intrarenal pressure was held at a lower level, in three experiments, by clamping the renal vein during systemic hypotension. A catheter was inserted through the right femoral vein and positioned in the vena cava at the level of the right renal vein. The inferior vena cava was tied around

the catheter below the level of the renal vein (IVCO 1, long bar at the bottom of Figure 8), which produced a marked decrease in venous return and hence a marked fall in the systemic blood pressure. After 2–3 minutes of equilibration, the inferior vena cava was also tied, just above the level of the renal vein, about 5 mm beyond the tip of the venous catheter, thus obstructing renal venous outflow (IVCO 2, short bar). We were thus able to monitor renal venous pressure during conditions of renal venous obstruction and low arterial pressure.

In one experiment (Fig. 8), the activity of two units was recorded simultaneously from the same nerve bundle. Unit 2 emitted a brief burst of impulses when the thread was tied around the inferior vena cava below the renal vein, presumably because of mechanical disturbance. The increase in venous pressure subsequent to the occlusion of the vena cava above the renal vein (IVCO 2) immediately activated unit 2; activity ceased upon the release of the occlusion and did not reappear during the ensuing renal artery occlusion. Its behavior was that of a mechanoreceptor sensitive to increments in venous pressure.<sup>4</sup> Unit 1 showed a clearly different response. It was activated after almost 2 minutes of elevated venous pressure and was again activated by the subsequent occlusion of the renal artery. Similar results were obtained from two other units. These data indicate that the effects observed during

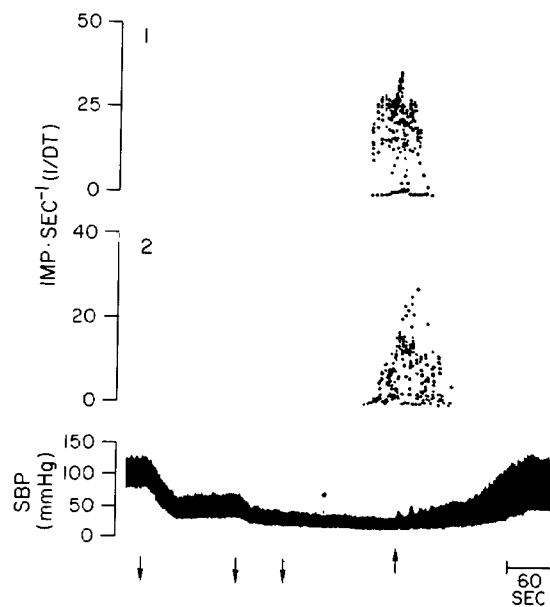


FIGURE 6 Effects of progressive systemic hypotension and, hence, reduction in renal blood flow, produced by hemorrhage. Traces as in Figure 3. Downward arrows indicate withdrawal of blood from a catheter positioned in the femoral vein. The upward arrow indicates the beginning of reinjection of blood. Two units became active when systolic blood pressure fell below 40 mm Hg. Their activation ceased during the recovery of systemic blood pressure.

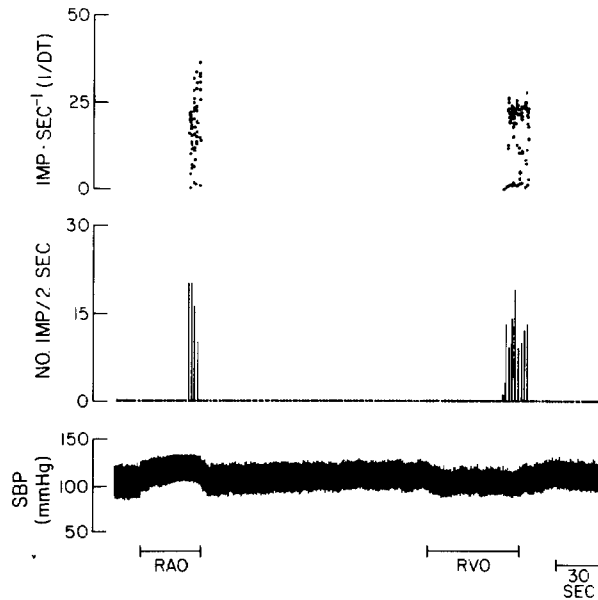


FIGURE 7 Effects of renal vein occlusion (RVO), performed at normal systemic arterial blood pressure, on a single unit sensitive to renal ischemia (RAO). Traces as in Figure 2. The unit was activated by a brief occlusion of the renal artery and by an occlusion of the renal vein. The latency of the response to RVO was longer (53 seconds) than the latency to RAO (32 seconds). Note also that the unit responded with a similar pattern of activation, i.e., bursts of impulses, to both RAO and RVO.

venous occlusion were due to renal ischemia and not to increments in intrarenal pressure.

The effects of prolonged venous occlusion were studied in four units (four experiments). Unlike the case with a prolonged arterial occlusion, the units studied were still active after 3, 4, 5, and 8 minutes of occlusion, respectively. The ischemia produced by venous stasis, therefore, has a more prolonged effect on renal afferent fibers than that produced by renal arterial occlusion.

#### Changes in Ureteral Pressure

At the beginning of each experiment, a 50-cm catheter was placed into the right ureter and positioned close to the pelvis of the kidney. It was raised on occasion, to increase ureteral pressure and to test for the presence of ureteral mechanoreceptors in the nerve filament from which we were recording. As a rule, they were very sensitive to small increments (2–5 cm H<sub>2</sub>O) in ureteral pressure and, hence, easily identified. Figure 9 shows the effect of raising the ureteral cannula on the activity of two units, which were not spontaneously active. At the first arrow (Fig. 9A), the ureteral catheter was raised above the kidney, and the urine level was maintained at 10 cm H<sub>2</sub>O. Unit 2 responded to this stimulus with an irregular discharge. At the second arrow, the ureteral catheter was raised further to increase hydrostatic pressure to 20 cm H<sub>2</sub>O. The record shown in Figure 9B was made 27 minutes

after the end of that in Figure 7A; ureteral hydrostatic pressure had been stable for 14 minutes at 40 cm H<sub>2</sub>O, and only unit 2 was active. An occlusion of the renal artery was initiated (RAO), which inhibited unit 2 and activated unit 1, 40 seconds later. On release of the occlusion, unit 1 became silent, whereas unit 2 progressively renewed its activity.

Ten units responsive to ischemia were studied during increments of ureteral pressure to 30–40 mm Hg, produced by backflow of urine into the kidney. None was activated.

Such increments may not have been transmitted to the renal tubules, for the outside pressure on the

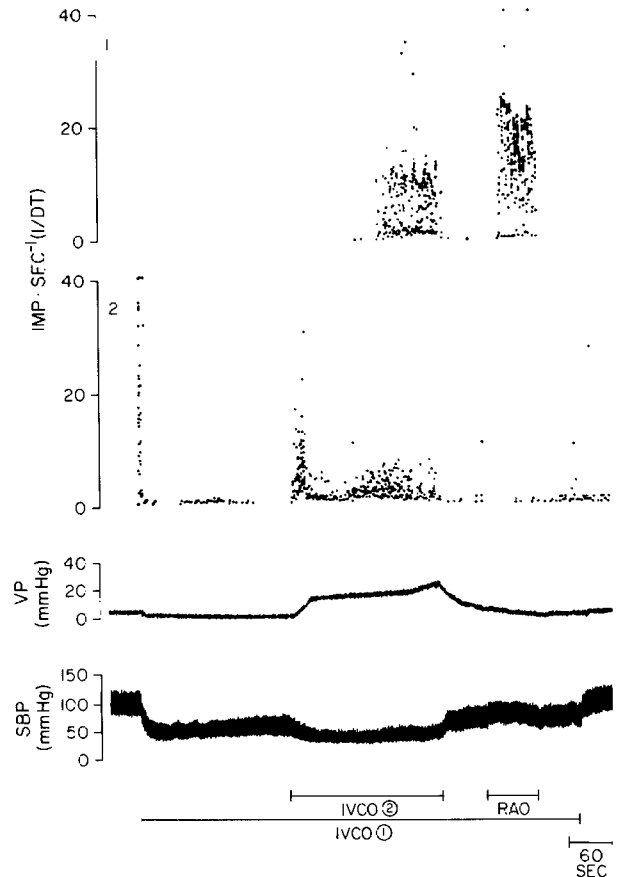


FIGURE 8 Effects of an increase in renal venous pressure at low systemic arterial blood pressure. From top to bottom, traces are: instantaneous firing rate of two units (1 and 2), venous pressure measured in the inferior vena cava at the junction of the renal vein (VP), and systemic arterial blood pressure (SBP). Unit 2 was activated momentarily when the inferior vena cava below the renal vein was tied (IVCO 1) and, subsequently, during the rise in venous pressure which followed the occlusion of the inferior vena cava above the renal vein (IVCO 2). The activation ceased at the release of IVCO 2. This unit was not activated during occlusion of the renal artery performed 1 minute after the release of IVCO 2. Unit 1 was activated 128 seconds after the beginning of the IVCO 2 and was markedly activated during the following RAO.

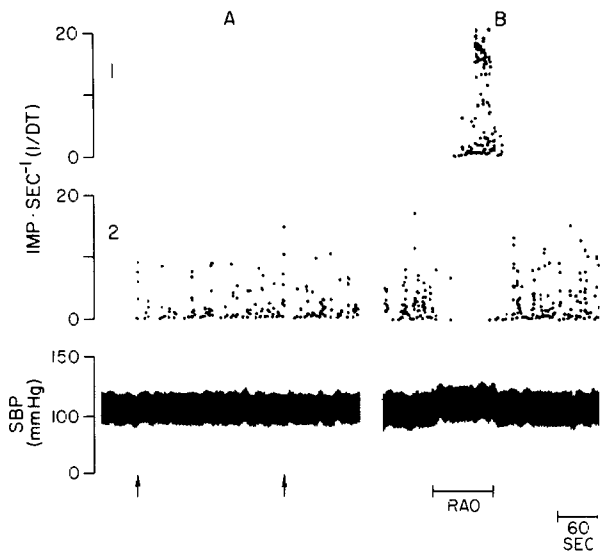


FIGURE 9 Effects of increments in ureteral pressure produced by raising the ureteral cannula. Spontaneously breathing rat. Traces from top to bottom are: instantaneous firing rate of two units (1 and 2) and systemic blood pressure. A: Unit 2 was activated by the initial increase in ureteral pressure to 10 cm H<sub>2</sub>O (first arrow) and slightly more by a second increase to 20 cm H<sub>2</sub>O (second arrow). B: Record taken 27 minutes after the end of A, when ureteral pressure had been stable for 14 minutes at 40 cm H<sub>2</sub>O and unit 2 had become even more active. The occlusion of the renal artery (RAO) inhibited unit 2, whereas unit 1, which was not activated by increments in ureteral pressure, showed a clear response.

papilla could have occluded the collecting ducts.<sup>11</sup> Consequently, in another group of rats (three experiments; three units) ureteral pressure was increased by rapid expansion of blood volume with isotonic saline (0.5 ml/min), after the ureteral catheter had been connected to a pressure transducer, to produce a more gradual and homogeneous distension of the renal tubules. Figure 10 shows the effect on a single unit sensitive to ischemia; the recording was made 22 minutes after the onset of the blood volume expansion, with ureteral pressure stabilized at 45 mm Hg. At the start of renal artery occlusion, ureteral pressure fell to 10 mm Hg. The afferent activity appeared with a 63-second latency and ceased at the release of the occlusion, whereas ureteral pressure progressively increased toward preocclusion levels. Two minutes after the release of occlusion, the pressure transducer was opened to air (upward arrow) and urine was allowed to flow freely out of the transducer. A renal artery occlusion repeated 90 seconds later, at zero ureteral pressure, revealed no marked difference in the response of this unit to renal ischemia. None of the three units studied was activated by increments in ureteral pressure up to 40–50 mm Hg produced by blood volume expansion, thus indicating that the units

sensitive to ischemia are not sensitive to slow or step-wise changes in ureteral pressure from 0 to 50 mm Hg. These experiments also indicate that the units responsive to ischemia are not affected by fast decreases or increases in intratubular pressure and, hence, are unaffected by the tubular collapse which occurs at the beginning of renal artery occlusion.

### Effects of Cyanide

Potassium and sodium cyanide are known to be potent stimulators of carotid body chemoreceptors. We therefore studied five units during injections of potassium and sodium cyanide (10 μg, 0.1 ml) into the descending aorta, above the level of the renal arteries; none of the five was activated. When 5 μg of sodium or potassium cyanide (0.03 ml) were injected directly into the renal artery, however, four of six units studied (four experiments) were activated. The same units showed intermittent bursts of activity after the needle's insertion, probably as a consequence of severely impaired renal blood flow. Conversely, similar amounts of isotonic saline injected directly into the renal artery always inhibited the activity of these units, as described earlier ("wash-out" effect).

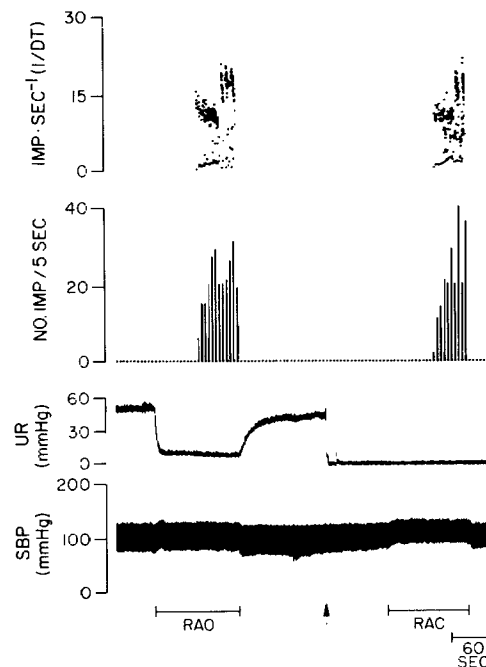


FIGURE 10. Effects of renal artery occlusion performed at high and low ureteral pressure. Traces from top to bottom are: instantaneous firing rate of single unit, number of spikes each 5 seconds, ureteral pressure (UR), and systemic blood pressure (SBP). The first occlusion of the renal artery (RAO) was done 22 minutes after the beginning of blood volume expansion with isotonic saline (0.5 ml/min). The unit was activated 63 seconds after the start of occlusion, and was also activated with a similar pattern during an occlusion performed at zero ureteral pressure.



## Discussion

Alterations in intrarenal pressure, which readily activate renal mechanoreceptors, did not affect the renal receptors that we have described. These receptors were activated only during systemic asphyxia and during markedly reduced renal blood flow produced by occlusion of the renal artery, occlusion of the renal vein, and severe hypotension. The stimulus responsible for their activation should therefore be found in the chemical alterations occurring in the kidney as a consequence of ischemia. Since nerve endings that respond to alterations of their chemical environment are termed chemoreceptors,<sup>12, 13</sup> the renal receptors responsive to ischemia also can be considered as renal chemosensitive nerve endings. For convenience they will be referred to as "R" chemoreceptors. This definition implies no functional similarity to arterial chemoreceptors (which would require demonstration of a specific sensitivity to changes in  $PO_2$ ,  $PCO_2$ , and pH of the arterial blood<sup>12, 13</sup>); it simply indicates chemical but not mechanical sensitivity.

The present work gives only preliminary indications concerning the nature of the chemical stimulus involved. Changes in tissue  $PO_2$ , for example, seem not to be of prime importance, inasmuch as the perfusion of the ischemic kidney with deoxygenated isotonic saline still produced a complete cessation of the afferent activity. This observation would be explicable if a substance released by the ischemic kidney or which accumulated at the receptor site were washed out during the perfusion. If this were the case, a complex control of the production and release of this substance by the ischemic kidney would need to be conjectured to explain the changes in latency during repeated ischemic episodes, the exhaustion of the discharge during prolonged renal artery occlusion, and the persistence of the activation during prolonged occlusion of the renal vein. Prostaglandins,<sup>14</sup> renin,<sup>15</sup> adenosine,<sup>16</sup> and bradykinin<sup>17</sup> all could be released by the ischemic kidney. A local accumulation of  $CO_2$  and changes in intrarenal pH,<sup>18</sup> moreover, cannot be discounted as possible factors. The response to asphyxia, however, is not clear evidence of a direct effect of hypercapnia. The activation always occurred during a fall in blood pressure and, as for hemorrhage, could have been due to a decrease in renal perfusion pressure.

Renal chemoreceptors clearly differ in their functional properties from arterial chemoreceptors. The latter always are spontaneously active,<sup>19</sup> respond to prolonged ischemia with an irregular and prolonged discharge,<sup>20</sup> and are markedly activated during perfusion of the carotid body with deoxygenated solutions.<sup>21</sup> Moreover, whereas cyanide always excites arterial chemoreceptors, it affects "R" chemoreceptors only when they are already spontaneously active. Although these differences cannot be explained, the answer may be found in differences in

the morphology of these two chemoreceptors, for the functional properties of arterial chemoreceptors are highly dependent on the structural relationships occurring between free nerve endings and surrounding glomus cells.<sup>22</sup>

Owing to the shortness of the nerves in our preparation the conduction velocity of renal nerve fibers could not be measured. The long duration of the action potentials (2–3 msec), which always exceeded that for afferent fibers responsive to increments in arterial blood pressure, suggests that the impulses from "R" chemoreceptors are conducted along unmyelinated nerve fibers. Electrophysiological and histological studies of the renal nerves of the rabbit have shown that the few myelinated axons, as against the large number of unmyelinated nerve fibers, carry impulses only from mechanosensitive nerve endings.<sup>8</sup> Although the total number of "R" chemoreceptors cannot be estimated from our results, we think that they represent a sizable population. In all the experiments, a large population of unitary potentials was activated in whole renal nerves by ischemia and, in some cases, we found small nerve bundles containing afferent fibers responsive only to ischemia.

The available anatomical data give no useful information about the morphology and location of renal sensory nerve endings. The renal innervation has been considered to be mainly efferent in function, and no anatomical studies have yet been attempted in order to differentiate between motor and sensory renal innervation.<sup>23–26</sup> The presence of nerve cells inside the renal parenchyma has been denied.<sup>23–26</sup>

## Physiological Role

The renal chemoreceptors were activated only when renal blood flow was markedly decreased, thus suggesting a limited role as an emergency system. It should be emphasized, however, that in our experimental conditions, we always isolated the experimental kidney from efferent nervous influences by cutting the greater splanchnic nerve and all other visible nerves joining the renal plexus. This was done specifically to avoid the possible influence on sensory nerve endings of neurally mediated alterations in renal function, as has been reported for the heart and cardiac receptors.<sup>27</sup> In an innervated kidney, the threshold pressure for excitation during hemorrhage, for example, could differ from the value we obtained, because hypotension elicits a marked renal vasoconstriction through activation of baroreceptor reflexes.

Renal chemoreceptors might be assumed to convey renal pain sensation. However, this seems improbable, because the functional properties of the "R" chemoreceptors are clearly different from those of cutaneous,<sup>28</sup> muscular,<sup>29</sup> and visceral receptors<sup>30</sup> involved in the sensation of pain.

Both hypotensive<sup>31</sup> and hypertensive<sup>32</sup> effects

have been reported to result from electrical stimulation of the central cut end of renal nerves. It is of interest, however, that in the most recent study,<sup>32</sup> the reflex hypertension and tachycardia caused by electrical stimulation of renal nerves could never be reproduced by stimuli known to affect the activity of intrarenal mechanoreceptors. The investigators therefore postulated that additional receptor groups, such as osmo- and chemoreceptors, may exist in the kidney. A similar conclusion was reached by MacFarlane<sup>33</sup> to explain a vasoconstrictor reno-renal reflex. The demonstration of renal chemoreceptors should help in attempting a more complete evaluation of the physiological role of renal receptors, by studying the reflex effects of selective stimulation of the different groups of renal sensory nerve endings.

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